Supporting Information

Novel Paeonol Derivatives Alleviate Lipid Accumulation in Low-dose Treatment

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Experimental

General Procedure

All reactions were carried out in oven-dried glassware (120 °C) under an atmosphere of nitrogen. Acetone, acetonitrile, ethanol, ethyl acetate and hexane from Mallinckrodt Chemical Co. were dried and distilled from CaH$_2$. 4-(2-Aminoethyl)morpholine, 1-(2-aminoethyl)piperazine, 1-(2-aminoethyl)piperidine, 1-(2-aminoethyl)pyrrolidine, benzylamine, 4-(2-chloroethyl)morpholine hydrochloride, 1-(2-chloroethyl)piperidine hydrochloride, 1-(2-chloroethyl)pyrrolidine hydrochloride, ethanolamine, ethylamine, ethylenediamine, hydrazine solution, hydroxylamine solution, isopropylamine, methylamine solution, paeonol (2'-hydroxy-4'-methoxyacetophenone), phenylhydrazine, potassium carbonate, propylamine were purchased from Sigma-Aldrich Chemical Co without further purification.

Analytical thin layer chromatography (TLC) was performed on precoated plates (silica gel 60 F-254), purchased from Merck Inc. Purification by gravity column chromatography was carried out by use of Merck Reagents Silica Gel 60 (particle size 0.063-0.200 mm, 70-230 mesh ASTM). $^1$H NMR spectra were obtained on a Bruker Avance 500 (500 MHz), Varian Unity-400 by use of chloroform-$d$ as solvent. $^1$H NMR chemical shifts were referenced to the CHCl$_3$ singlet (7.24 ppm). $^{13}$C NMR spectra were obtained on a Bruker Avance 500 (125 MHz), Bruker AM-400* and Varian MR-400 by use of chloroform-$d$ as solvent. $^{13}$C chemical shifts were referenced to the center of the CDCl$_3$ triplet (77.0 ppm). Multiplicities were recorded by the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; J, coupling constant (hertz). High-resolution mass spectra were obtained by means of a FINNIGAN/MAT-95XL mass spectrometer. High-performance liquid chromatography (HPLC) analyses were carried out by Agilent 1100 series system with CNW Athena C18 column (120 Å, 4.6 mm × 250 mm, 5 μm) and UV detection at 254 nm. A mixture of 20% DI water in acetonitrile was used as eluent and flow rate was at 0.5mL/min.
Synthesis and characterization

Paeonol imine derivatives 4

A solution containing Paeonol (1.00 equiv.) and appropriate primary amine (1.20 equiv.) in ethanol (25.0 mL) was refluxed for 12.0 h or reacted at r.t. for 12.0 h (PB1 and PB2). The residue obtained after concentration was purified by column chromatography over silica gel using AcOEt and hexane as eluent or rinse with ethanol.

**(E)-5-methoxy-2-(1-(methylimino)ethyl)phenol (4a).** Yellow crystals (57%); $^1$H NMR (400 MHz, CDCl$_3$): $\delta$ = 7.30 (d, $J_{AB}$ = 9.2 Hz, 1H), 6.29 (d, $J_{CD}$ = 2.8 Hz, 1H), 6.18 (dd, $J_{CD}$ = 2.8 Hz, $J_{AB}$ = 9.2 Hz, 1H), 3.77 (s, 3H), 3.24 (s, 3H), 2.32 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 173.15, 172.36, 164.40, 129.36, 110.99, 104.89, 102.49, 54.88, 32.54, 13.40; HRMS-EI m/z for C$_{10}$H$_{13}$NO$_2$ calcd 179.2165; found 179.0943.

![Image of (E)-5-methoxy-2-(1-(methylimino)ethyl)phenol (4a)](image)

**(E)-2-(1-(ethylimino)ethyl)-5-methoxyphenol (4b).** Yellow crystals (72% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.30 (d, $J_{AB}$ = 9 Hz, 1H), 6.28 (d, $J_{CD}$ = 2.5 Hz, 1H), 6.18 (dd, $J_{CD}$ = 2.5 Hz, $J_{AB}$ = 9 Hz, 1H), 3.77 (s, 3H), 3.55 (q, 2H), 2.33 (s, 3H), 1.36 (t, $J = 7.5$ Hz, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta$ = 173.43, 170.72, 164.52, 129.45, 110.96, 105.07, 102.65, 55.01, 40.76, 14.97, 13.67; HRMS-EI m/z for C$_{11}$H$_{15}$NO$_2$ calcd 193.2432; found 193.1100.

![Image of (E)-2-(1-(ethylimino)ethyl)-5-methoxyphenol (4b)](image)

**(E)-5-methoxy-2-(1-(propylimino)ethyl)phenol (4c).** Yellow crystals (79% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ = 7.30 (d, $J_{AB}$ = 9 Hz, 1H), 6.28 (d, $J_{CD}$ = 2.5 Hz, 1H), 6.18 (dd, $J_{CD}$ = 2.5 Hz, $J_{AB}$ = 9 Hz, 1H), 3.77 (s, 3H), 3.47 (t, $J = 7.5$ Hz, 2H), 2.32 (s, 3H), 1.75 (m, 2H), 1.04 (t, $J = 7.5$ Hz). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the same
compound reported in the literature.  

**(E)-2-(1-(isopropylimino)ethyl)-5-methoxyphenol (4d).** Yellow crystals (60% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.30$ (d, $J_{AB} = 9$ Hz, 1H), 6.28 (d, $J_{CD} = 2.5$ Hz, 1H), 6.18 (dd, $J_{CD} = 2.5$ Hz, $J_{AB} = 9$ Hz, 1H), 4.00 (m, 1H), 3.77 (s, 3H), 2.35 (s, 3H), 1.32 (d, $J = 6.5$ Hz). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the same compound reported in the literature.

**(E)-5-methoxy-2-(1-(2-(piperidin-1-yl)ethylimino)ethyl)phenol (4e).** Yellow crystals (59% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.32$ (d, $J_{AB} = 9$ Hz, 1H), 6.30 (d, $J_{CD} = 2.5$ Hz, 1H), 6.20 (dd, $J_{CD} = 2.5$ Hz, $J_{AB} = 9$ Hz, 1H), 3.77 (s, 3H), 3.64 (t, $J = 7$ Hz, 2H), 2.68 (t, $J = 7$ Hz, 2H), 2.47 (m, 4H), 2.33 (s, 3H), 1.58 (m, 4H), 1.43 (m, 2H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta =$ 172.08, 171.16, 164.31, 129.47, 111.52, 105.25, 102.51, 58.57, 55.10, 54.80, 44.90, 25.90, 24.14, 14.17; HRMS-EI m/z for C$_{16}$H$_{24}$N$_2$O$_2$ calcd 276.3754; found 276.1843.

**(E)-5-methoxy-2-(1-(2-morpholinoethylimino)ethyl)phenol (4f).** Yellow crystals (65% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.34$ (d, $J_{AB} = 9$ Hz, 1H), 6.30 (d, $J_{CD} = 2.5$ Hz, 1H), 6.23 (dd, $J_{CD} = 2.5$ Hz, $J_{AB} = 9$ Hz, 1H), 3.77 (s, 3H), 3.72 (t, $J = 4$ Hz, 4H), 3.72 (t, $J = 7$ Hz, 2H), 2.72 (t, $J = 7$ Hz, 2H), 2.54 (t, $J = 4$ Hz, 4H), 2.33 (s, 3H); $^{13}$C NMR (125 MHz, CDCl$_3$):
\( \delta = 171.38, 171.08, 164.18, 129.41, 111.64, 105.31, 102.36, 66.86, 58.15, 55.08, 53.73, 44.61, 14.21; \) HRMS-EI m/z for \( \text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}_{3} \) calcd 278.3481; found 278.1636.

\begin{figure}
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\caption{(E)-5-methoxy-2-(1-(2-(piperazin-1-yl)ethyl)iminoo)ethyl)phenol (4g). Orange crystals (76\% yield); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta = 7.31 \) (d, \( J_{AB} = 9 \) Hz, 1H), 6.30 (d, \( J_{CD} = 2.5 \) Hz, 1H), 6.20 (dd, \( J_{CD} = 2.5 \) Hz, \( J_{AB} = 9 \) Hz, 1H), 3.75 (s, 3H), 3.62 (t, \( J = 7 \) Hz, 2H), 2.88 (t, \( J = 4.5 \) Hz, 4H), 2.69 (t, \( J = 7 \) Hz, 2H), 2.49 (t, \( J = 4.5 \) Hz, 4H), 2.31 (s, 3H). Its spectroscopic characteristics in \(^{13}\)C NMR are consistent with those of the same compound reported in the literature\(^2\).

\begin{figure}
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\caption{(E)-5-methoxy-2-(1-(2-(pyrrolidin-1-yl)ethyl)iminoo)ethyl)phenol (4h). Orange viscous oil (71\% yield); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \( \delta = 7.33 \) (d, \( J_{AB} = 9 \) Hz, 1H), 6.31 (d, \( J_{CD} = 2.5 \) Hz, 1H), 6.21 (dd, \( J_{CD} = 2.5 \) Hz, \( J_{AB} = 9 \) Hz, 1H), 3.77 (s, 3H), 3.68 (t, \( J = 7 \) Hz, 2H), 2.86 (t, \( J = 7 \) Hz, 2H), 2.61 (t, \( J = 5.5 \) Hz, 4H), 2.33 (s, 3H), 1.79 (t, \( J = 5.5 \) Hz, 4H); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)): \( \delta = 171.69, 171.25, 164.31, 129.50, 111.64, 105.36, 102.48, 55.77, 55.14, 54.46, 46.63, 23.51, 14.21; \) HRMS-EI m/z for \( \text{C}_{15}\text{H}_{22}\text{N}_{2}\text{O}_{2} \) calcd 262.3487; found 262.1680.
(E)-5-methoxy-2-(1-(2-phenylhydrazono)ethyl)phenol (4i). Orange crystals (75% yield); 
$^1$H NMR (500 MHz, CDCl$_3$): $\delta = 12.83$ (s, 1H), 7.33-7.27 (m, 2H), 7.08-6.89 (m, 4H), 6.52-6.44 (m, 2H), 3.80 (s, 3H), 2.31 (s, 3H). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the same compound reported in the literature$^3$.

(E)-2-(1-(benzylimino)ethyl)-5-methoxyphenol (4j). Yellow crystals (70% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.38$-$7.26$ (m, 6H), 6.34 (d, $J_{CD} = 2.4$Hz, 1H), 6.26 (dd, $J_{CD} = 2.8$ Hz, $J_{AB} = 9.2$ Hz, 1H), 4.75 (s, 2H), 3.78 (s, 3H), 2.36 (s, 3H). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the same compound reported in the literature$^4$.

(E)-1-(2-hydroxy-4-methoxyphenyl)ethanone oxime (4k). White crystals (78% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.31$ (d, $J_{AB} = 8.5$ Hz, 1H), 6.48 (d, $J_{CD} = 2.5$ Hz, 1H), 6.45 (dd, $J_{CD} = 2.5$ Hz, $J_{AB} = 8.5$ Hz, 1H), 3.79 (s,3H), 2.30 (s, 3H). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the same compound reported in the literature$^5$.

(E)-2-(1-(2-hydroxyethylimino)ethyl)-5-methoxyphenol (4l). Yellow crystals (65% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.27$ (d, $J_{AB} = 9$ Hz, 1H), 6.27 (d, $J_{CD} = 2.5$ Hz, 1H), 6.20 (dd, $J_{CD} = 2.5$ Hz, $J_{AB} = 9$ Hz, 1H), 3.94 (t, $J = 5$ Hz, 2H), 3.77 (s, 3H), 3.68 (t, $J = 5$ Hz, 2H), 2.33 (s, 3H). Its spectroscopic characteristics in $^{13}$C NMR are consistent with those of the
same compound reported in the literature\textsuperscript{6}.

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\end{center}

6,6'-\textit{(1E,1'E)-1,1'-(hydrazine-1,2-diylidene)bis(ethan-1-yl-1-ylidene)bis(3-methoxyphenol)} (5a). Yellow crystals (30\% yield); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta = 7.52\) (d, \(J_{AB} = 8.5\) Hz, 2H), 6.50 (d, \(J_{CD} = 2.5\) Hz, 2H), 6.48 (dd, \(J_{CD} = 2.5\) Hz, \(J_{AB} = 8.5\) Hz, 2H), 3.83 (s,6H), 2.51 (s, 6H). Its spectroscopic characteristics in \textsuperscript{13}C NMR are consistent with those of the same compound reported in the literature\textsuperscript{7}.

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6,6'-\textit{(1E,1'E)-1,1'-(ethane-1,2-diylbis(azan-1-yl-1-ylidene))bis(ethan-1-yl-1-ylidene)bis(3-methoxyphenol)} (5b). Yellow crystals (33\% yield); \textsuperscript{1}H NMR (500 MHz, CDCl\textsubscript{3}): \(\delta = 7.35\) (d, \(J_{AB} = 9\) Hz, 2H), 6.34 (d, \(J_{CD} = 2.5\) Hz, 2H), 6.27 (dd, \(J_{CD} = 2.5\) Hz, \(J_{AB} = 9\) Hz, 2H), 3.90 (s,4H), 3.76 (s, 6H), 2.32 (s, 6H). Its spectroscopic characteristics in \textsuperscript{13}C NMR are consistent with those of the same compound reported in the literature\textsuperscript{6}.

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\textit{o-Alkylation Paeonol derivatives 6a–c}

A solution containing Paeonol (1.00 equiv.), potassium carbonate (3.00 equiv.) and appropriate ammonium chloride (1.5 equiv.) in acetone (30.0 mL) was refluxed for 12.0 h.
Then the mixture was extracted with dichloromethane for three times, and dried over MgSO₄. After concentration of the solvent, the residue was purified by column chromatography over silica gel using methanol and dichloromethane as eluent.

1-(4-methoxy-2-(2-(piperidin-1-yl)ethoxy)phenyl)ethanone (6a). Orange crystals (90% yield); ¹H NMR (500 MHz, CDCl₃): δ = 7.81 (d, J_AB = 9 Hz, 1H), 6.50 (dd, J_CD = 2 Hz, J_AB = 9 Hz, 1H), 6.43 (d, J_CD = 2 Hz, 1H), 4.15 (t, J = 6.5 Hz, 2H), 3.82 (s, 3H), 2.80 (t, J = 6.5 Hz, 2H), 2.59 (s, 3H); ¹³C NMR (100 MHz, CDCl₃): δ = 197.70, 164.43, 160.01, 132.12, 121.12, 105.55, 99.01, 66.14, 57.43, 55.51, 54.89, 31.83, 25.42, 23.70; HRMS-EI m/z for C₁₆H₂₃NO₃ calcd 277.3601; found 277.1645.

1-(4-methoxy-2-(2-morpholinoethoxy)phenyl)ethanone (6b). Yellow crystals (90% yield); ¹H NMR (500 MHz, CDCl₃): δ = 7.81 (d, J_AB = 8.5 Hz, 1H), 6.51 (dd, J_CD = 2 Hz, J_AB = 8.5 Hz, 1H), 6.42 (d, J_CD = 2 Hz, 1H), 4.14 (t, J = 5.5 Hz, 2H), 3.83 (s, 3H), 3.70 (t, J = 4.5 Hz, 4H), 2.83 (t, J = 5.5 Hz, 2H), 2.59 (s, 3H), 2.54 (t, J = 4.5 Hz, 4H); ¹³C NMR (100 MHz, CDCl₃): δ = 197.73, 164.40, 160.12, 132.06, 121.39, 105.33, 99.10, 66.92, 66.13, 57.41, 55.51, 53.98, 32.02; HRMS-EI m/z for C₁₅H₂₁NO₄ calcd 279.3328; found 279.2413.
1-(4-methoxy-2-(2-(pyrrolidin-1-yl)ethoxy)phenyl)ethanone (6c). Orange viscous oil (83% yield); $^1$H NMR (500 MHz, CDCl$_3$): $\delta = 7.79$ (d, $J_{AB} = 8.5$ Hz, 1H), 6.50 (dd, $J_{CD} = 2$ Hz, $J_{AB} = 8.5$ Hz, 1H), 6.43 (d, $J_{CD} = 2$ Hz, 1H), 4.18 (t, $J = 6$ Hz, 2H), 3.81 (s, 3H), 2.98 (t, $J = 6$ Hz, 2H), 2.65 (m, 4H), 2.57 (s, 3H), 1.80 (m, 4H); $^{13}$C NMR (125 MHz, CDCl$_3$): $\delta = 197.61$, 164.30, 160.19, 132.44, 121.19, 105.28, 98.87, 67.74, 55.35, 54.582, 31.91, 29.55, 23.44; HRMS-EI $m/z$ for C$_{15}$H$_{21}$NO$_3$ calcd 263.3334; found 263.1465.

Standard procedure for the synthesis of aryl-sulfonate from peaconol with benzenesulfonyl chloride derivatives 7. To a reaction vessel containing peaconol (1.00 equiv.), benzenesulfonyl chloride derivatives (1.20 equiv.) and potassium carbonate (2.00 equiv.) in 20.0 mL acetone was stirred and refluxed for 12.0 hours. It was quenched with water and removed acetone under reduced pressure. The residue was extracted with ethyl acetate (3 × 30 mL). The combined organic layer were washed with brine, dried over MgSO$_4$, filtered, and concentrated under reduced pressure. The crude product was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products. All the products with purify >95.0% was check by HPLC.

4’-Methoxy-2’-[phenylsulfonyl]oxy]acetophenone (7a). A orange viscous oil (83% yield); IR (ATR, cm$^{-1}$) 3069 (w), 3012(w), 2841 (w), 1683 (m), 1607 (s), 1376 (s), 1257 (s), 1195 (s), 1120 (s), 1063 (s), 952 (m), 794 (s), 686 (s); $^1$H NMR (500 MHz, CDCl$_3$): $\delta$ 7.83 (dd, $J_{AB}=8.25$ Hz, $J_{CD}=1$ Hz, 2H), 7.67–7.65 (m, 2H), 7.52 (t, $J=7.5$ Hz,2H), 6.81 (dd, $J_{EF}=9$ Hz, $J_{GH}=2.5$ Hz, 1H), 6.57 (d, $J=2.5$ Hz,1H), 3.73 (s, 3H), 2.43 (s, 3H); $^{13}$C NMR (125 MHz,
CDCl₃): δ 196.1, 163.1, 148.9, 135.0, 134.6, 131.9, 129.3, 128.5, 125.7, 113.0, 108.5, 55.7, 30.3; HRMS (EI) calculated for C₁₅H₁₄O₅S, 306.0562, found 306.0556. Its spectroscopic are consistent with those of the same compound reported in our another manuscript.³

2′-[4-Fluorophenylsulfonyl]oxy]-4′-methoxy-acetophenone (7b). A white solid (82% yield); IR (ATR, cm⁻¹) 2962 (w), 2924 (w), 1680 (s), 1609 (w)m 1591 (m), 1491 (m), 1377 (s), 1258 (s), 1239 (s), 1151 (m), 951 (s), 791 (s); ¹H NMR (500 MHz, CDCl₃): δ 7.89–7.86 (m, 2H), 7.67 (d, J=9 Hz, 2H), 7.21 (t, J=8 Hz, 2H), 6.83 (dd, JAB=8.5 Hz, JCD=2.5 Hz, 1H), 6.65 (d, J=2.5 Hz, 1H), 3.79 (s, 3H), 2.44 (s, 3H); ¹³C NMR (125 MHz, CDCl₃): δ 195.9, 163.1, 148.5, 132.0, 131.5, 131.4, 125.5, 116.7, 116.6, 112.8, 108.8, 55.7, 30.0; HRMS (EI) calculated for C₁₅H₁₃FO₅S, 324.0468, found 324.0458. Its spectroscopic are consistent with those of the same compound reported in our another manuscript.³

2′-[4-Chlorophenylsulfonyl]oxy]-4′-methoxy-acetophenone (7c). A white solid (75% yield); IR (ATR, cm⁻¹) 3019 (w), 2922 (w), 2897 (w), 1680 (s), 1608 (s), 1566 (m), 1414 (m), 1256 (s), 1238 (m), 1183 (s), 1090 (s), 952 (s), 871 (s), 622 (s); ¹H NMR (500 MHz, CDCl₃): δ 7.77 (d, J=8.5 Hz, 2H), 7.66 (d, J=9 Hz, 1H), 7.49 (d, J=9 Hz, 2H), 6.82 (dd, JAB=9 Hz, JCD=2.5 Hz, 1H), 6.61 (d, J=2.5 Hz, 1H); ¹³C NMR (125 MHz, CDCl₃): δ 195.9, 163.1, 148.5, 141.4, 133.5, 132.1, 130.0, 129.6, 125.5, 112.9, 108.8, 55.8, 30.1; HRMS (EI) calculated for
C_{15}H_{13}ClO_5S, 340.0172, found 340.0170. Its spectroscopic are consistent with those of the same compound reported in our another manuscript.\(^8\)

\[\text{2'}-[(4-	ext{Bromophenylsulfonyl})\text{oxy}]\text{-4'}-	ext{methoxy-acetophenone (7d).} \quad \text{A white solid (75\% yield); IR (ATR, cm}^{-1}\text{)} 3093 (w), 2951 (w), 2850 (w), 1680 (s), 1609 (s), 1370 (s), 1256 (s), 1183 (m), 1150 (s), 952 (s), 798 (s), 609 (s); ^1H NMR (500 MHz, CDCl}_3\text{): } \delta 7.70-7.64 (m, 5H), 6.81 (dd, } J_{AB}=8.5 \text{ Hz, } J_{CD}=2 \text{ Hz, 1H}), 6.59 (d, } J=2 \text{ Hz, 1H}), 3.75 (s, 3H), 2.42 (s, 3H); ^{13}C \text{ NMR (125 MHz, CDCl}_3\text{): } \delta 195.8, 163.1, 148.4, 134.0, 132.6, 132.0, 130.0, 129.9, 125.4, 112.8, 108.7, 55.7, 30.0; \text{ HRMS (EI) calculated for C}_{15}H_{13}BrO_5S, 383.9967, found 383.9674. Its spectroscopic are consistent with those of the same compound reported in our another manuscript.\(^8\)}

\[\text{4'}\text{-Methoxy-2'}-[(\rho\text{-tolylsulfonyl})\text{oxy]}\text{acetophenone (7e).} \quad \text{A yellow solid (77\% yield); IR (ATR, cm}^{-1}\text{)} 2922 (w), 2862 (w), 2844 (w), 1681 (s), 1609 (m), 1368 (s), 1322 (m), 1257 (s), 1238 (m), 1193 (s), 1152 (s), 971 (s), 784 (s), 717 (s); ^1H NMR (500 MHz, CDCl}_3\text{): } \delta 7.67-7.62 (m, 3H), 7.28 (d, } J=8 \text{ Hz, 2H}), 6.77 (dd, } J_{AB}=8.5 \text{ Hz, } J_{CD}=2.5 \text{ Hz, 1H}), 6.55 (d, } J=2 \text{ Hz, 1H}), 3.70 (s, 3H), 2.41 (s, 3H), 2.40 (s, 3H); ^{13}C \text{ NMR (125 MHz, CDCl}_3\text{): } \delta 196.0, 163.0, 148.9, 145.9, 131.9, 131.8, 129.8, 128.4, 125.6, 112.7, 108.5, 55.6, 30.2, 21.6; \text{ HRMS (EI) calculated for C}_{16}H_{16}O_5S, 320.0718, found 320.0718. Its spectroscopic are consistent}
with those of the same compound reported in our another manuscript.\(^8\)

**4′-Methoxy-2′-[(4-methoxyphenylsulfonyl)oxy]-acetophenone (7f).** A white solid (81% yield); IR (ATR, cm\(^{-1}\)) 2972 (w), 2848 (w), 1667 (m), 1595 (m), 1564 (m), 1496 (m), 1412 (s), 1268 (s), 1172 (s), 835 (s), 780 (s); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.68 (d, \(J=8.5\) Hz, 2H), 7.61 (d, \(J=9\) Hz, 1H), 6.91 (d, \(J=8.5\) Hz, 2H), 6.75 (dd, \(J_{AB}=8.5\) Hz, \(J_{CD}=1.5\) Hz, 1H), 6.53 (t, \(J=2.5\) Hz, 1H), 3.81 (s, 3H), 3.69 (s, 3H), 2.41 (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 196.0, 164.3, 163.0 148.9, 131.8, 130.7, 125.9, 125.6, 114.4, 112.6, 108.5, 55.7, 55.6, 30.2; HRMS (EI) calculated for C\(_{16}\)H\(_{16}\)O\(_6\)S, 336.0668, found 336.0672. Its spectroscopic are consistent with those of the same compound reported in our another manuscript.\(^8\)

**4′-Methyl-2′-[(4-nitrophenylsulfonyl)oxy]-acetophenone (7g).** A yellow solid (78% yield); IR (ATR, cm\(^{-1}\)) 3105 (w), 3072 (w), 2992 (w), 2853 (w), 1671 (s), 1600 (s), 1528 (s), 1364 (s), 1354 (s), 1313 (s), 1193 (s), 1120 (s), 1059 (s), 933 (m), 870 (m), 803 (s), 747 (m), 658 (s); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 8.35 (dd, \(J_{AB}=7\) Hz, \(J_{CD}=2\) Hz, 2H), 8.08 (dd, \(J_{AB}=7\) Hz, \(J_{CD}=2\) Hz, 2H), 7.67 (d, \(J=9\) Hz, 1H), 6.85 (dd, \(J_{EF}=8.5\) Hz, \(J_{GH}=2.5\) Hz, 1H), 6.67 (d, \(J=2.5\) Hz, 1H), 3.79 (s, 3H), 2.41 (s, 3H); \(^13\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 195.6, 163.2, 151.0, 147.9, 140.7, 132.3, 130.0, 125.1, 124.3, 112.9, 109.2, 55.8, 29.7; HRMS (EI) calculated for C\(_{15}\)H\(_{13}\)NO\(_7\)S, 351.0413, found 351.0412. Its spectroscopic are consistent with those of the
same compound reported in our another manuscript.  

\[
\text{MeO}_2\text{O}
\]

\[
\text{MeS}_2\text{O}_2\text{N}
\]

2’-[(4-Aminophenylsulfonyl)oxy]-4’-methoxy-acetophenone (7h). 7g (100.0 mg, 0.295 mmol) was dissolved in 10.0 mL ethanol. And the reaction mixture was added 10% Pd/C (7.6 mg). Then the reaction mixture was stirred at room temperature for 12.0 hours. The mixture was filtered, and the filtrate was purified by column chromatography on silica gel (ethyl acetate and hexane as eluent) to give desired products. A yellow solid (86% yield); IR (ATR, cm\(^{-1}\)) 3473 (w), 3356 (w), 3239 (w), 2923 (s), 2853 (s), 1595 (s), 1464 (s), 1347 (s); \(^1\)H NMR (500 MHz, CDCl\(_3\)): \(\delta\) 7.66 (d, \(J=9\) Hz, 1H), 7.52 (d, \(J=7\) Hz, 2H), 6.78 (dd, \(J_{AB}=9\) Hz, \(J_{CD}=3\) Hz, 1H), 6.64–6.60 (m, 3H), 4.34 (b, 2H), 3.76 (s, 3H), 2.46 (s, 3H); \(^1\)C NMR (125 MHz, CDCl\(_3\)): \(\delta\) 196.7, 163.2, 152.3, 149.5, 131.8, 130.9, 125.9, 122.0, 113.8, 112.9, 108.6, 55.7, 29.7; HRMS (EI) calculated for C\(_{15}\)H\(_{15}\)NO\(_5\)S, 321.0671, found 321.0675. Its spectroscopic data are consistent with those of the same compound reported in our another manuscript.  

\[
\begin{align*}
\text{MeO}_2\text{O} & \xrightarrow{\text{H}_2, \text{1 atm}} \text{MeS}_2\text{O}_2\text{N}
\end{align*}
\]
$^1$H NMR and $^{13}$C NMR of 4a
$^1$H NMR and $^{13}$C NMR of 4b
$^1$H NMR and $^{13}$C NMR of 4e
$^{1}$H NMR and $^{13}$C NMR of 4f
$^1$H NMR and $^{13}$C NMR of 4h

![NMR Spectrum](image)

**NMR Details**

- **Chemical Shifts**:
  - $^1$H NMR: Various ppm values indicated
  - $^{13}$C NMR: Various ppm values indicated

**Spectral Data**

- **Sample Information**:
  - Sample ID: 2015
  - Method: n

- **Processing Information**:
  - Processing ID: 2015
  - Comments: n

- **Additional Details**:
  - ppm: Various values indicated
  - Other parameters: Various values indicated
$^1$H NMR and $^{13}$C NMR of 6a
$^{1}$H NMR and $^{13}$C NMR of 6b
$^1$H NMR and $^{13}$C NMR of 6c
$^1$H NMR and $^{13}$C NMR of 6d
HPLC analysis of 4a

![HPLC analysis of 4a](image)

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAu *s</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.896</td>
<td>0.1163</td>
<td>1577.702</td>
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</table>

HPLC analysis of 4b

![HPLC analysis of 4b](image)

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

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<tr>
<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAu *s</th>
<th>Area %</th>
</tr>
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HPLC analysis of 4e

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
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<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAu *s</th>
<th>Area %</th>
</tr>
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<tr>
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<td>6.192</td>
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</table>

HPLC analysis of 4f

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

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<th>Width (min)</th>
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<th>Area %</th>
</tr>
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<tr>
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<td>2</td>
<td>7.898</td>
<td>0.1551</td>
<td>64.37434</td>
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</table>
HPLC analysis of 4h

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
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<th>Peak #</th>
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<th>Width (min)</th>
<th>Area mAU *s</th>
<th>Area %</th>
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</thead>
<tbody>
<tr>
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<td>7.84914</td>
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<td>0.1647</td>
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</table>

HPLC analysis of 6a

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
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<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAU *s</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.990</td>
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</table>
HPLC analysis of 6b

![HPLC peak chart for 6b](image)

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
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<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
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</table>

HPLC analysis of 6c

![HPLC peak chart for 6c](image)

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAu *s</th>
<th>Area %</th>
</tr>
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<tbody>
<tr>
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<td>7.796</td>
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<td>26.32948</td>
<td>0.4306</td>
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<tr>
<td>---</td>
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<td>------</td>
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<tr>
<td>3</td>
<td>19.484</td>
<td>1.1721</td>
<td>5905.6766</td>
<td>96.5771</td>
</tr>
</tbody>
</table>
HPLC analysis of 7a

VWD1 A, Wavelength = 254 nm; eluent: 20% DI water in acetonitrile; flow rate = 0.5mL/min

<table>
<thead>
<tr>
<th>Peak #</th>
<th>Ret Time (min)</th>
<th>Width (min)</th>
<th>Area mAu *s</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.514</td>
<td>0.5247</td>
<td>936.00690</td>
<td>2.2541</td>
</tr>
<tr>
<td>2</td>
<td>7.933</td>
<td>0.3902</td>
<td>40588.0</td>
<td>97.7459</td>
</tr>
</tbody>
</table>
Materials and Methods

Reagents

Paeonol (2’-Hydroxy-4’-methoxyacetophenone, purity >99% HPLC) was from Sigma Chemical (St. Louis, MO, USA), Dil-labeled oxLDL was from Biomedical Technologies (Stoughton, MA, USA).

Cell Culture

Murine macrophage J774.A1 cells (American Type Culture Collection, TIB-67) were cultured in RPMI 1640 medium (HyClone, Logan, UT) supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/ml), and streptomycin (100 μg/ml) (HyClone).

Fluorescent assay

Macrophages were pre-treated with various concentrations of paeonol and paeonol derivatives for 12h, then equilibrated with Dil-oxLDL for an additional 18h in the presence of drugs (0, 0.1, 1, 10, 100 μg/ml). Cells were washed and lysates were analyzed by fluorometry (Molecular Devices) at 514nm excitation and 550nm emission.

Statistical analysis

Data are presented as mean standard error of the mean (SEM) from five independent experiments. Mann–Whitney test was used to compare two independent groups and Kruskal–Wallis followed by the Bonferroni post hoc analyses for multiple groups. SPSS v20.0 (SPSS, Inc., Chicago, IL) was used for analysis. Differences were considered statistically significant at P<0.05.

MTT assay

Cell viability was examined by methyl thiazolyltetrazolium (MTT) assay. Macrophages were
pre-treated with various concentrations of paconol and paconol derivatives for 18h. Then 5 mg/ml MTT were added into culture medium at 37°C for 10-15 mins. The resulting crystals were dissolved in isopropanol and analyzed by Microplate Reader (Molecular Devices) at 570nm.
### Table S1 Water Solubility of Paeonol derivatives

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$S_w$ (mg/mL)</th>
<th>Compounds</th>
<th>$S_w$ (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paeonol</td>
<td>0.524</td>
<td>4i</td>
<td>N/A</td>
</tr>
<tr>
<td>4a</td>
<td>1.950</td>
<td>4j</td>
<td>0.071</td>
</tr>
<tr>
<td>4b</td>
<td>1.937</td>
<td>4k</td>
<td>0.296</td>
</tr>
<tr>
<td>4c</td>
<td>0.862</td>
<td>4l</td>
<td>1.765</td>
</tr>
<tr>
<td>4d</td>
<td>1.704</td>
<td>5a</td>
<td>N/A</td>
</tr>
<tr>
<td>4e</td>
<td>1.795</td>
<td>5b</td>
<td>N/A</td>
</tr>
<tr>
<td>4f</td>
<td>2.436</td>
<td>6a</td>
<td>0.210</td>
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<tr>
<td>4g</td>
<td>6.579</td>
<td>6b</td>
<td>0.856</td>
</tr>
<tr>
<td>4h</td>
<td>2.100</td>
<td>6c</td>
<td>1.158</td>
</tr>
</tbody>
</table>

### Table S2 IC$_{50}$ value of compound 4k, 7b, 7c, 7d, 7f and 7h.

<table>
<thead>
<tr>
<th>Compounds</th>
<th>IC$_{50}$ (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4k</td>
<td>64.8</td>
</tr>
<tr>
<td>7b</td>
<td>15.5</td>
</tr>
<tr>
<td>7c</td>
<td>3.8</td>
</tr>
<tr>
<td>7d</td>
<td>5.0</td>
</tr>
<tr>
<td>7f</td>
<td>0.8</td>
</tr>
<tr>
<td>7h</td>
<td>2.8</td>
</tr>
</tbody>
</table>

### Figure S1 MTT assay result of compound 4k, 7b, 7c, 7d, 7f and 7h.

### Notes and references


